CRYOSURGERY AND PERIPHERAL NERVE FUNCTION

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INTRODUCTION

In 1914 Jehn reported a case of tetanus in which the phrenic nerves had been divided in an attempt to overcome rigidity of the chest and diaphragm, and so facilitate artificial respiration. Stimulated by this work, Trendelenburg (1917) published the results of a series of animal experiments in which the phrenic nerves of the dog had been frozen with ethyl chloride. He correctly reasoned that interruption of nerve conduction by cold would be followed by regeneration and functional recovery.

With the recent advances in cryogenic technology, the destruction of tissue by freezing has become an established therapeutic technique in the treatment of neoplasia (Gage *et al.*, 1965). Radical surgery for neoplastic disease frequently results in mutilation and functional deficits if the growth has involved such structures as major blood vessels or nerves. It was assumed that similar penalties would be incurred by the use of cryosurgery in these situations. Less information is available on the behaviour of peripheral nerves in a similar context although sporadic clinical reports suggest that frozen nerves both in continuity and after suture may undergo a surprisingly efficient reconstitution. If such clinical impressions were substantiated, then/

then cryosurgery may become the treatment of choice in those situations where the use of conventional surgery carries a high risk of residual, and often incapacitating, neurological damage.

With these considerations in mind, an experimental model is reported in which peripheral nerve function could be studied after exposure to temperatures of the order utilised in cryosurgery today.

MATERIALS AND METHODS

The combined median - ulnar nerve of the rat - supplies the flexor group of "forearm" muscles and was chosen because of its accessability and easily observed motor effect. Forty Sprague-Dawley rats were used for the investigations which were carried out under ether anaesthesia.

The nerve was exposed as it emerged from the axilla into the proximal portion of the limb and its diameter was measured through a dissecting microscope with a calibrated graticule incorporated in the eyepiece. Stimulation was effected by a pair of silver wire electrodes 0.5 mm in diameter, spaced 2 mm apart, and hooked around the nerve. A supramaximal stimulus was employed with a pulse duration of 100 m.sec from a double pulse unit (2 Giro, Electro-Physiological Instruments, E.P.I., Edinburgh). Single compound muscle action potentials were recorded by Copeland-Davies clip electrodes penetrating the exposed forearm flexor muscles (Copeland and Davies, 1964), and observed at an oscilloscope sweep speed/

speed of 2 m.sec/cm. The recording system incorporated a pre-amplifier with a gain of 100 and was used at a time constant of 25 m.sec. This was included in a Tele-equipment oscilloscope (Model D52), while photographic recording was carried out using a Tektronix storage oscilloscope 502 with a Shackman polaroid camera, (Fig. 1).

The nerve was frozen by a 2.2 mm cryoprobe coupled to a Linde-Cooper cryosurgery unit CE-2A (Union Carbide Corporation) (Fig. 2) achieving a pre-set minimum temperature of -100°C. The probe was applied directly to the nerve at a point distal to the stimulating electrodes and the temperature maintained for one minute, (Fig. 3). Readings were taken from thermocouples placed on the nerve surface opposite to that in contact with the cryoprobe and from fixed points distal and proximal to the centre of the cryolesion. The diameter of the cryolesion was measured through the dissecting microscope immediately before thawing.

Six animals were submitted to nerve stimulation electromyography before and immediately after freezing. The remaining animals were observed for clinical return of function and were evaluated at intervals increasing from 30 minutes to 60 days, with stimulation electromyography prior to sacrifice. Control recordings were taken at this time from the unfrozen nerve - muscle preparation of the opposite limb. The animals were examined daily for clinical evedence of nerve regeneration, and a comparison was made between "frozen"/

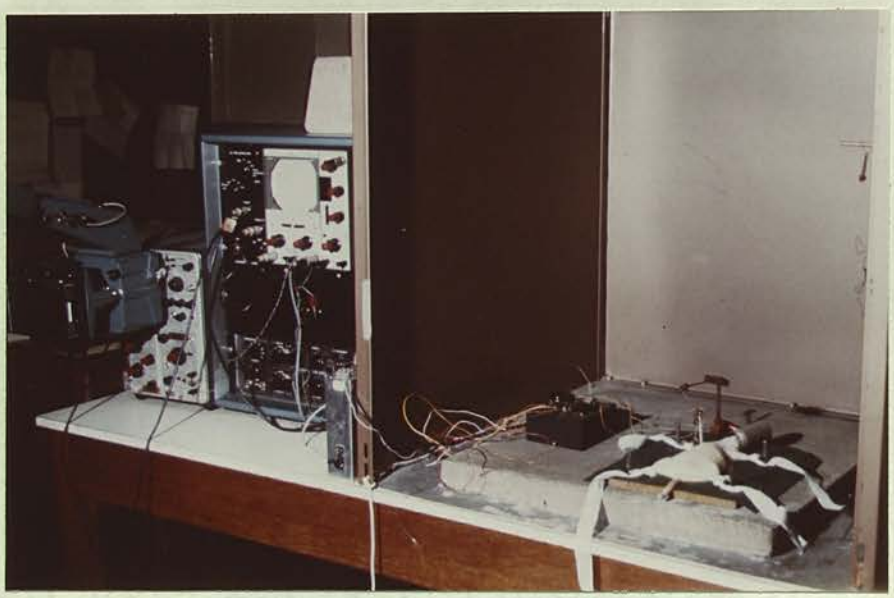

Fig. 1

Apparatus for electromyographical recording showing stimulator, oscilloscope and tetrex recording equipment.

Fig. 2

The Linde-Cooper Cryosurgical Unit.

Fig. 3a

Median-ulnar nerve complex of rat forelimb hooked over stimulating electrodes.

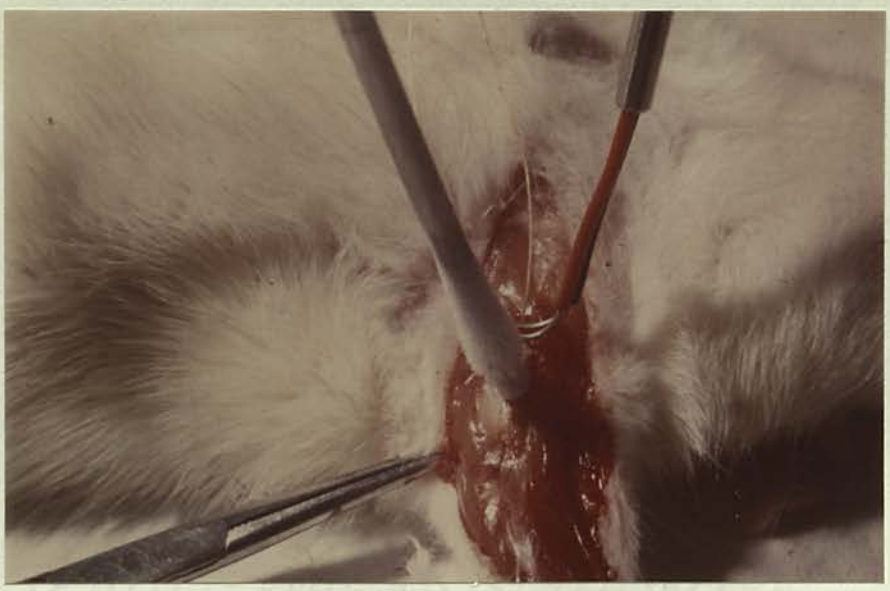

Fig. 3b

Cryoprobe in place during freezing of nerve complex.

"frozen" and "unfrozen" limbs in respect of:-

(i) the use of the limb in the animal's routine caged existence

(ii) the ability of the "forearm" muscles to actively flex the "wrist"

(iii) the power of the claws to grip a wire mesh when the animal was suspended by the tail.

Biopsies from each animal were taken at sacrifice of the previously frozen segment of nerve and from the regions immediately proximal and distal to this area. These were processed for light microscopic examination and stained with H & E, reticulin techniques (Lendrum and Gordon Sweet), trichrome preparations (Masson and Van Gieson), Marchi's technique, osmium fixation and luxol fast blue - P.A.S.

RESULTS

A. Clinical Observations

The mean diameter of the combined nerve was 0.92 mm prior to freezing and the subsequent cryolesion measured at least 0.85 cm in diameter.

On the basis of the criteria described, a return of function to the affected muscles was first apparent on the 14th day after freezing. Function increased steadily thereafter in all animals until/

until by the 25th day it became impossible to appreciate any differences between the two forelimbs.

B. Nerve Stimulation Electromyography

1. Acute experiments. Six animals were evaluated immediately before and after freezing. No evidence of nerve-conduction could be obtained in the immediate post-thaw period (Figs. 4 and 5).

2. Short term experiments. Eight animals were evaluated at 30 minutes, 1, 2, 3, 6, 9, 12 and 24 hours respectively, after freezing. No evidence of intact nerve function was obtained although direct stimulation of the "forearm" muscle demonstrated no impairment of muscle viability.

3. Long term experiments. The remaining animals were sacrificed daily from the first to the 10th post-operative day and thereafter at 3-day intervals until day 58. The return of function noted clinically was confirmed by stimulation electromyography in that nerve conduction reappeared in all animals by the 14th day (Fig. 6).

C. Microscopic Examination

The histological changes which occur in the peripheral nerve subsequent to freezing are essentially those of neuropraxia (Sneddon) or perhaps more accurately the second degree of injury described by Sunderland. Immediately after thawing the segment of nerve frozen shows no gross structural change but over the ensuing 24 hours/

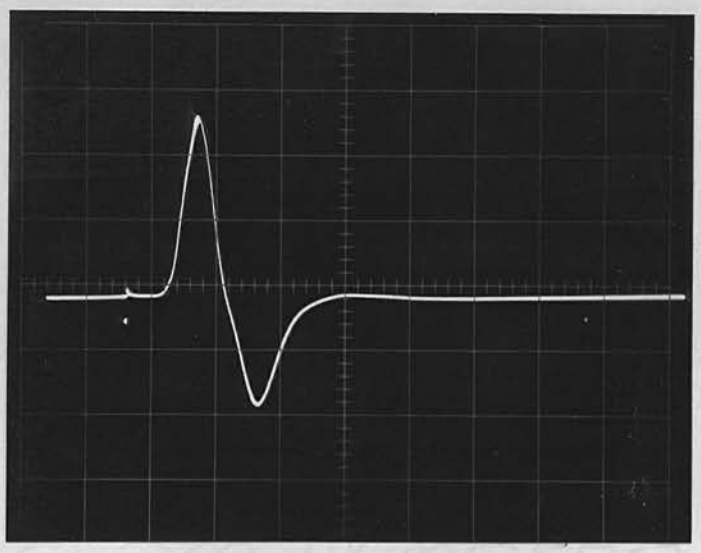

Fig. 4

Normal compound electromyographic tracing recorded from rat forearm flexor muscles prior to freezing.

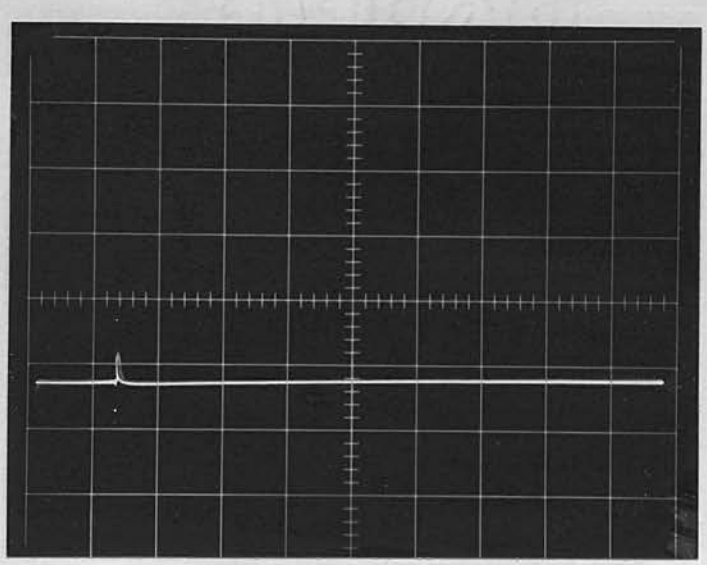

Fig. 5

Electromyographic tracing immediately following freezing process showing no electrical activity.

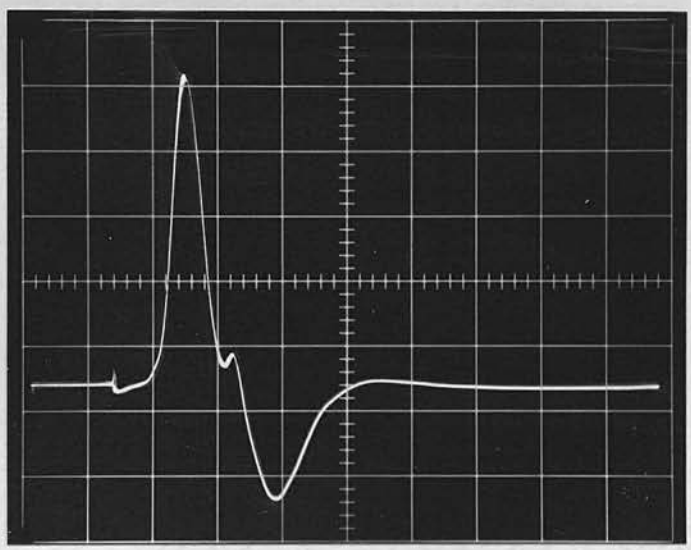

Fig. 6

Electromyographic tracing at day 20 showing return of normal electromyographic activity.

24 hours evidence of cellular damage appears. All components of the nerve are involved and the various sheaths as well as the axons are eventually devitalised. Wallerian and retrograde degeneration occur synonymously as in more orthodox forms of nerve injury (Figs. 7 and 8).

At this stage the degenerative processes take place within connective tissue sheaths (epi-, peri- and endoneural coverings and the neurilemma) which although devitalised maintain their integrity as uninterrupted collagenous tubes. Characteristic of tissue killed by cold, the inflammatory reaction is minimal.

By the 12th day Schwann cell proliferation was a prominent feature and this appeared more orderly than usually seen after nerve injury and was again associated with minimal inflammatory reaction. Revitalisation commenced at the same time in the remaining sheaths and now nuclei appeared in the perisiting connective tissue sheaths, although hyperplastic fibroplasia was not a feature.

Axon sprouting was already underway and proceeds in a more orderly fashion than with other forms of nerve injury.

In summary the sequence of histological events closely parallels that seen in neuropraxia with the differences:-

(i) of minimal inflammatory reaction and almost insignificant fibroplasia

(ii) of minimal interference with all connective tissue sheaths which consequently remain in relatively undistorted/

Fig. 7

Nerve bundle distal to freeze at day 8 showing pale, demyelinated nerve fibres.

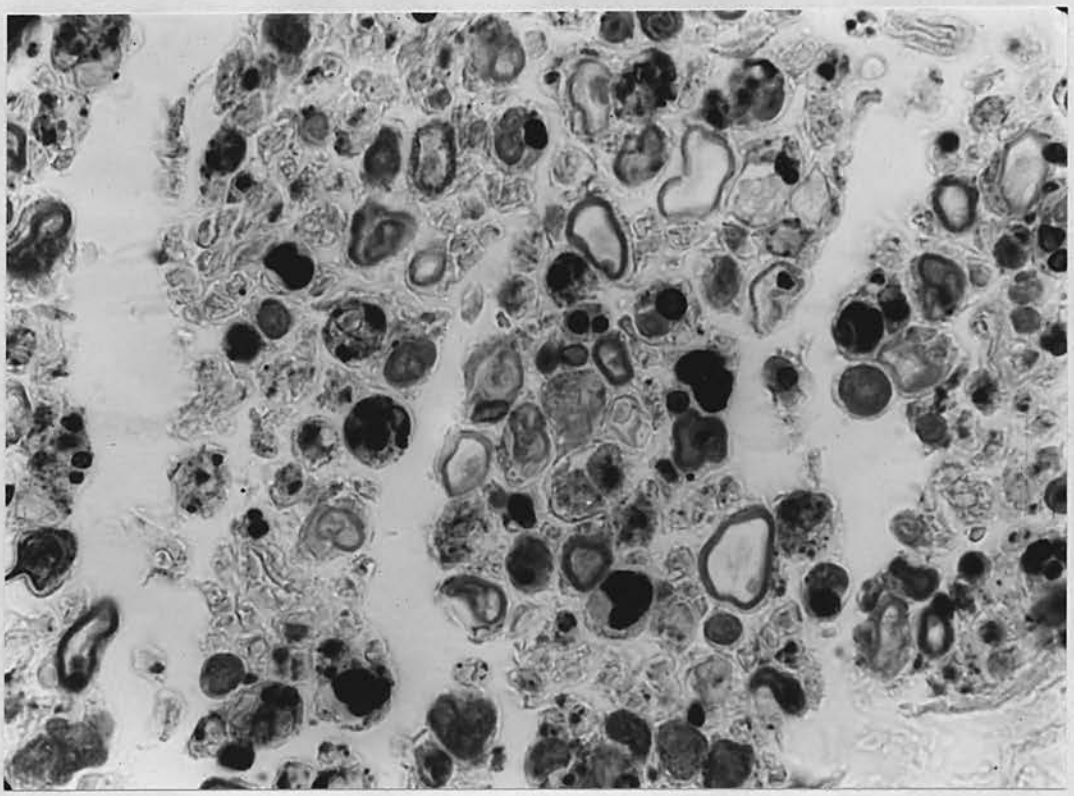

Fig. 8

High power view of Fig. 7 showing evidence of Wallerian degeneration note myelin droplets.

undistorted positions.

Histological evidence of regeneration is as shown in Fig. 9.

DISCUSSION

The effects of cooling on peripheral nerve function have been extensively reported in the literature. Trendelenburg (1917) regarded cold as the most "gentle" form of nerve injury and used a temperature of approximately -7°C to produce a degree of structural damage compatible with subsequent regeneration. Blackwood and Russell (1943) confined their attentions to the clinical syndrome of "immersion foot" and studied nerve function after exposure to saline at 4 - 5°C. The classical work of Denny-Brown and his colleagues (1945) clarified the pathological changes which follow cold injury by exposing peripheral nerves to saline at -4° to +3°C, or a carbon dioxide spray. These workers made no attempt to measure the actual temperatures achieved in the nervous tissue. We have been unable to find relevant information in the literature which correlates low temperatures of the order utilised in modern cryosurgery with resultant nerve injury. For this reason a controlled temperature of -100°C was employed in the present study to reproduce the conditions within a cryolesion.

The nerve lesion produced by freezing may be regarded as the second degree injury described by Sunderland (1968). This constitutes a failure of the axon to survive below the level of injury/

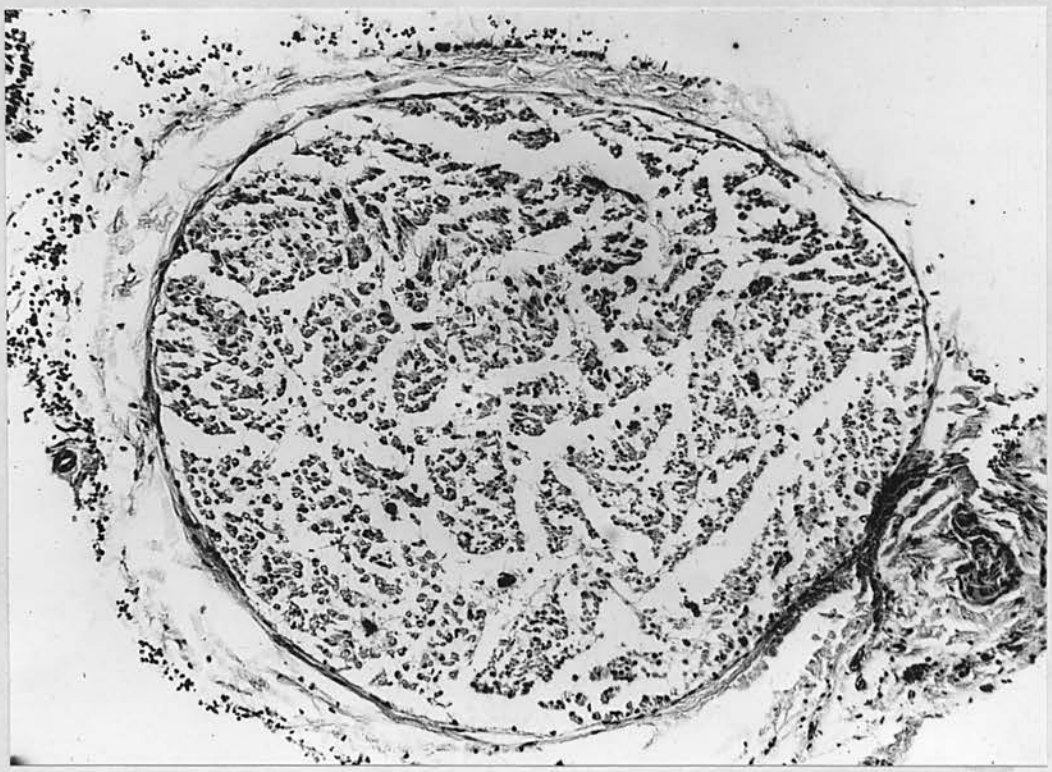

Fig. 9

Nerve bundle distal to freeze at day 30 showing complete regeneration and return of normal nerve architecture.

injury and for a short distance above this point, and results in the classical changes of Wallerian and retrograde degeneration. In the more advanced third degree injury, there is additional disorganisation of the internal connective tissue components of the funiculus, and although the perineurium remains intact, the continuity of endoneurial tubes and Schwann complexes is destroyed. This has an adverse effect on nerve repair because of the inevitable intrafunicular fibrosis which diverts, delays and distorts, subsequent axon growth.

One of the properties of cryocoagulated tissue left in situ is its relatively inert biological behaviour in producing only a minimal inflammatory response from the adjacent body tissues (Fraser, 1967). Thus when peripheral nerves are incorporated in a cryolesion, not only is there preservation of intrafunicular tubular morphology but there is a characteristically slight inflammatory response. Both conditions favour unimpeded axon regeneration within their original endoneurial tubes and meticulous re-alignment with end organs.

It is generally accepted that the rate of axonal growth is more rapid after second degree injury than after nerve suture. The prognosis of increasingly distal lesions improves in that muscle changes consequent upon denervation have little time to develop before re-innervation occurs. This must be tempered with the knowledge that the rate of axon growth declines as the distance between/

between the axon tip and its parent cell body increases (Sunderland, 1968). In the model described, the times noted for recovery are compatible with an injury of the second degree type.

Axon growth and fibre maturation are separate, although closely related, features of regeneration. Similarly, fibre maturation and functional recovery are slightly separated in time. It follows that detection of the onset of motor recovery may vary chronologically with the method of assessment employed. Direct stimulation of the nerve frequently produces muscular contraction which antedates the return of voluntary function (Sunderland, 1947). After crushing rabbit nerves Gutmann and Young (1944) observed growing nerve fibres in close proximity to the end plates by the 12th day; on the 18th day they obtained the first response to nerve stimulation and by the 23rd day noted return of reflex function. We first obtained a positive electromyographic response to nerve stimulation at 14 days after freezing and this was associated with the simultaneous appearance of voluntary function. Although it is difficult to quantitate the degree of functional return we concluded that full clinical recovery was present 25 days after freezing, as there was no appreciable difference between the performance of "frozen" and "unfrozen" limbs. Stimulation electromyography is also a relatively coarse index of functional return due to the difficulties in achieving constant placing of the electrodes and stimulator. Despite the inability to grade recovery/

recovery there is no doubt that full return of function occurred in terms of clinical performance and a classical stimulation electromyogram. Such a course of events again supports our supposition that the freezing trauma to nerves constitutes a second degree injury.

The freezing experiments of Denny-Brown and his colleagues (1945) differ from our own in this respect. These authors suggest that a third degree injury results from freezing the cat sciatic nerve. Attempts to compare the investigations may not be valid as Denny-Brown did not measure the temperature of the nerve, varied the duration of freezing from 15 seconds to 5 minutes and, the length of nerve frozen from 10 to 15 mm. In addition only 4 of the 22 animals used were allowed to survive the freezing episode beyond 21 days. Complete functional recovery was observed in the 2 animals permitted to survive 99 days, despite the histological features of a third degree injury.

The actual mechanism of the cryoinjury remains largely speculative. It is certain that vascular stasis and ischaemia provide a major contribution, but it is also possible that freezing may have a direct cryolytic effect on myelin and disrupt the enzyme systems responsible for nerve sustenance. The ability of small nerves to obtain nutrition by direct diffusion processes further complicates the problem. Certainly, cryodestruction in other tissues is thought to be mediated predominately through ischaemia (Fraser/

(Fraser and Gill, 1967 and Walder). Mobilisation of a nerve will damage its nutrient arteries but it has been shown that an effective collateral circulation is rapidly established. Experimental ligation of regional nutrient arteries over considerable lengths of a nerve trunk do not impair the structure and function of nerve fibres, or their regeneration after crush injury (Blunt, 1960). In the present experiments, a normal response to stimulation was present after the nerve has been mobilised, indicating that a significant vascular insufficiency was not present prior to freezing.

A number of workers have studied the functional changes associated with cooling of sensory nerves (Douglas and Malcolm, 1955 and Torrance and Whitteridge, 1948), and demonstrated a variable susceptibility in the different types of nerve fibres. While these observations cannot be completely explained in terms of fibre size (Blackwood, 1944), Denny-Brown and his colleagues (1945) reported that large and medium-sized myelinated fibres were selectively damaged by cooling, in contrast to fine non-myelinated fibres which were relatively resistant. A selective response is unlikely after exposure to the lower temperatures utilised in cryotherapy where an all or nothing lethal effect is anticipated. There is no evidence to suggest that the response of sensory nerves will differ significantly from that of their motor counterparts.

The/

The crucial factor which influences the quality of regeneration is the preservation of the intrafunicular connective tissue architecture, in particular the integrity of the individual endoneurial tubes (Fig. 10). The minimal inflammatory response to cryocoagulated tissue has prompted work by Sakurai and his colleagues (personal communication) which suggests that the results of nerve suture might be improved by the freezing of nerve proximal to the suture line. The ensuing Wallerian degeneration distal to the frozen site, causes anappreciable delay before regenerating fibres arrive at the suture line. Sakurai reasons that any interference with axon realignment from the anastomotic in inflammation will be minimised. Of current interest is the work of Jacoby and his colleagues (Neurosurgical Clinic, Munich University) on the use of transplanted freeze-dried cadavar nerves. The freeze drying is thought to eliminate antigenicity and therefore graft rejection, while maintaining sufficient structural integrity to provide excellent guide lines for regenerating axon sprouts. Their preliminary results are most encouraging and the technique offers exciting potential in the future management of peripheral nerve injuries.

With respect to clinical application of the experimental findings it is possible that cryosurgery may find a place in the treatment of parotid cancer. The conventional treatment of such

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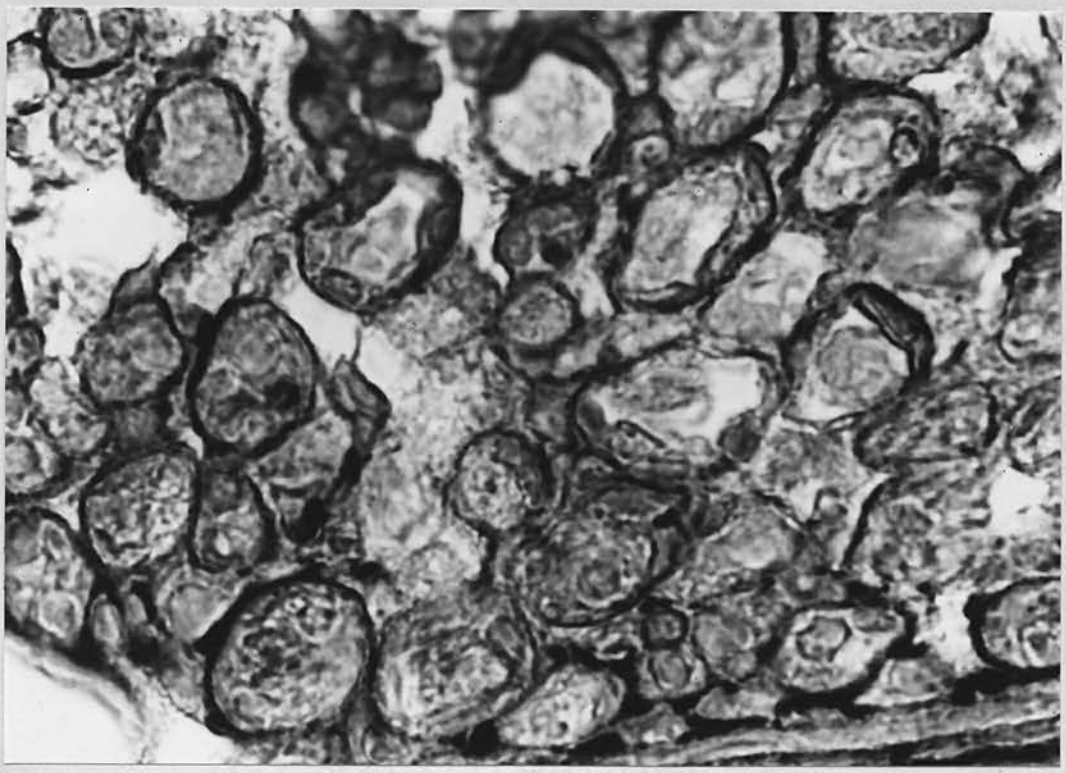

Fig. 10

High power view of frozen area of nerve at the 8th day following freeze.
Graham and Sweet connective tissue stain showing intact endoneurial sheath.

a tumour involves surgical excision with subsequent loss of the facial nerve function on that side; were the tumour to be destroyed by freezing and regeneration of the facial nerve to occur through the frozen area the morbidity of the operation would be much reduced. With this in mind a series of normal rabbit parotid glands have been subjected to freezing (as shown in Fig. 11), and evaluation of the degree of regeneration and return of nerve function is at present being carried out.

SUMMARY

1. The effect of cryosurgical temperatures on peripheral nerves is described in an experimental model.

2. The observations indicate that a second degree type of nerve injury is produced and explain the complete return of function after freezing.

3. The clinical implications of these findings are discussed.

ACKNOWLEDGMENTS

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Fig. 11

Exposure of left rabbit parotid gland with cryoprobe in place prior to freezing.

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